# Report of Foreign Exploration for Potential Biological Control Agents for Rush Skeletonwood, 2007

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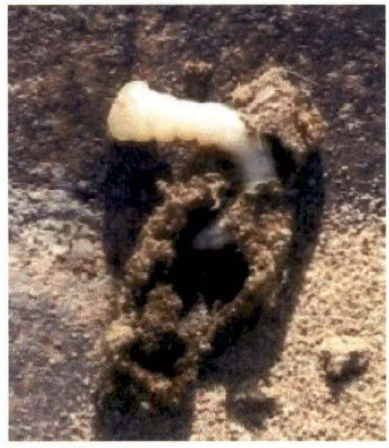

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**Report of Foreign Exploration for Potential Biological Control Agents for Rush Skeletonweed, 2007**

Prepared by G.P. Markin

December 10, 2007

**Introduction:**

In 2007, the US Forest Service Rocky Mountain Research Station (RMRS) continued its effort to coordinate a foreign exploration program to identify, test, and ship to quarantine in the US potential biocontrol agents of rush skeletonweed. During this period, approximately $50,000 of RMRS Bozeman biocontrol laboratory funds were supplied to cooperators in Europe, primarily by grants to the ARS Foreign Laboratories Program, EBCL Laboratory in Montpellier, France, and grants to MSU Bozeman. The state of Idaho's rush skeletonweed task force, provided an additional $25,000 to support the work of Massimo Cristofaro of BBCA in Rome, Italy. Progress in 2007 was less than expected due to a number of problems in transferring the funds and weather. This report summarizes progress made in 2007.

**Reports of ongoing studies**

Greece: In 2007, a series of field tests of three potential biocontrol agents of skeletonweed were to be conducted at Thessaloniki, Greece using cages covering plots containing both skeletonweed and selected non-target plants. Cages and plants were set up in spring of 2007, but when it came time to collect the insects from the field that would be introduced into the cages to see if they oviposited on or their progeny fed on the non-target plants, a major problem occurred. In 2007, Greece experienced its worst drought and series of wildfires in its recent history. Many potential collecting sites were lost and most of the natural enemies to be studied could not be collected or completed their development much earlier than expected. As a result, the planned field cage host testing studies were inconclusive (see Attachment 1).

Bulgaria: Drs. Ivanka Lecheva and Massimo Cristofaro continued their studies of two potential biocontrol agents.

*Schinia cognata:* *Schinia cognata* has been identified as the most promising natural enemy of rush skeletonweed in Bulgaria. Studies of its field biology, developing methods for rearing, and feeding tests on non-target plants were continued in 2007. This insect continues to look very promising.

*Smyrna nervosa:* *Smyrna nervosa* is a foliage feeding caterpillar that in the field appears to be specific to rush skeletonweed and our cooperators continued their study of its biology and feeding host range. However, from laboratory studies they have concluded that it is probably not a potential biocontrol agent, but more studies are needed (see Appendix 2).

Russia: Despite a major problem in getting funding to our Russian cooperators in time for the 2007 field season, they continued a series of laboratory and field studies in Russia, Kazakhstan, and Armenia on the root feeding beetle *Sphenoptera foveola.* Tests used adult beetles placed on caged rush skeletonweed and several related species of plants to see if they would feed and oviposite. Preliminary results indicate that *S. foveola* will only

attack various species of *Chondrilla* and not attack any of the non-target species tested. *S. foveola* therefore still appears to be a promising potential biocontrol agent. The studies will be continued in 2008 (see Attachment 3).

**New studies initiated in 2007**

Taxonomy of the genus *Sphenoptera*: To date, we have identified populations of two species of the root boring beetles *Sphenoptera* that may attack rush skeletonweed, *S. foveola* and *S. clarensces*. However, as we progress with our studies we are finding a major problem concerning the taxonomy of these two insects and the complex of closely related species. Since any introduction into the United States of a new biocontrol agent must be accompanied by a positive identification and a key that can be used by homeland security introduction laboratories personnel to confirm identification of future shipments, we realized that this could be a major problem in working with these two insects. Accordingly, when the chance occurred to support a graduate student interested in *Sphenoptera* taxonomy working under the direction of taxonomist Mike Ivie at Montana State University, we arranged to support the student through a joint venture agreement between RMRS and MSU. The student, Crystal Maier, made one trip to Europe in late fall 2007 to review taxonomic collections of these insects and meet beetle taxonomists Mark Volkovitsh of the Russian Academy of Science and Mark Kalashian of the Armenian Academy of Science. She returned with the first series of specimens for her to study. Crystal will return in '08 to Europe to continue to study specimens in collections and work with these two taxonomists. She will also join Margarite Volkovitsh to observe *S. foveola* in the field in Russia, and with Massimo Cristofaro to observe *S. clarensces* in Turkey to obtain information on their biology which she can use to support her final taxonomic description.

**Shipments to the United States**

In 2007, Javid Kashefi at the USDA-EBCL substation laboratory in Thessaloniki, Greece, made three shipments of rush skeletonweed insects to Jeff Littlefield's insect containment facility in Bozeman, Montana. Two shipments were of *Bradyrrhoa gilveolla*, which we needed to maintain genetic diversity in our laboratory colony. The third shipment was of a new insect, the flower feeding moth *Schinia cognata*.

**Conclusion**

Despite an increase in funding to support our foreign programs, we did not show any major progress or breakthroughs. The work conducted was an unexciting, unglamorous, series of monotonous studies needed to obtain the basic information on five natural enemies of *Chondrilla* in Europe. However, eventually these studies will generate the information we need to either drop these insects as having no potential or to move them to the next step of testing, quarantine studies in the United States. Fires, drought, and administrative problems in transferring the funding slowed down the work but much of the money which was not spent in 2007 hopefully can be carried over to 2008.

# 2007 Report of Studies of Potential Biological Control agents for *Chondrilla juncea* in Greece

Javid Kashefi

September 12, 2007

## 1. Chondrilla juncea

1.1. **Sawfly rearing** (Hymenoptera: Tenthredinidae): On June 04, 2007 one of our Sawfly locations near Xanthi, E. Greece was visited and 25 small larvae were collected and transferred individually to the laboratory in transparent plastic tubes. They were then places in plastic Petri dishes and were fed daily with fresh Chondrilla plants. During the first week plants were simply cut and placed in Petri dishes. During the second week, plants were sprayed with 2% Bonomyl and then placed in Petri dishes. All the larvae seemed to be very healthy and grow rapidly during these two weeks. After this period, the larvae stopped feeding and died very fast. The color of dead larvae changed from white to green. Petri dishes were kept on working benches in laboratory with temperatures between 25°C and 38°C. Samples of the dead larvae have been preserved on silica gel and will be sent to EBCL / France, for possible identification of the pathogen causing the mortality. I will also try to see if it is possible to isolate the pathogen here in Greece and then send it for identification.

1.2. **Brachycoleus decolour** (Hemiptera: Miridae): On May 07, 2007 during a trip to Lake Prespa, the main source of B. declour, 60 immature stages of this Miridae were collected and brought back to the laboratory. They were then released in 3 bugdorm cages (60 x 60 x 60 cm) and were provided every 4$^{th}$ or 5$^{th}$ day with fresh cuttings of: 1. Chondrilla juncea, 2. Cichorium intybus, 3. Lactuca viminea, 4. Lactuca sativa, 5. leaves of Taraxacum officinalis. During these tests, the first one or 2 days after the change of plants we would observe no feeding on any plants then C. juncea but after that feeding signs were observed on C. intybus and L. sativa. Testing was terminated after 20 days when most of the larvae died probably from extreme heat.

1.3. **Schinia cognata** (Lepidoptera: Noctuidae): On 24 July 2007 the first 30 larvae of S. cognate were collected after 2 days of search from one of our best sites on road Ptolemaida to Florina, NW Greece. Eight of these larvae were most probably F1 generation since they were collected only from the feeding sign I saw on the small buds where they had entered and they were less than 1 mm long. Before transferring these larvae to 2 x 2 x 2 meters field cage, because of an unusual hot summer and concessive heat wave, I decided to monitor the temperatures inside the cage. Because of bad air circulation and hot weather, the temperature would raise up to 59°C. Because of unusual high temperatures, I decided to do the host specificity test in 2 BugDorm cages in the laboratory under more suitable conditions. To have the test done under more real and reliable conditions, the temperature was kept around 30 to 32°C and at night the laboratory windows were opened and the temperature would go down to 25 - 27°C.

These larvae were provided with flower and seed heads of Chondrilla juncea, Cichorium intybus, Lactuca viminea, Lactuca sativa, and leaves of Taraxacum officinalis since I did not have any flowering plants available. Separate bouquets from each test plant was made and put in a plastic container with water to keep

them fresh. They were than placed in the cages in a manner that plants would touch each other and make it easier for the larvae to move from one plant to another. Bouquets were changed every second day and the old plants were controlled for feeding signs. When choosing fresh seed heads we were trying to have only healthy and good ones in order to be able to observe any feedings when changing them. Three time daily the behavior of larvae were observed and if any feeding on other plants except C. juncea was observed, this would be recorded.

On 04 August another 20 larger larvae were collected from the same location and added to a 3ed BugDorm cage. These were fed only with Chondrilla seeds heads for obtaining pupae for a possible oviposition test incase I could not send them to US.

All larvae fed only on C. juncea seed heads and did not attack any other plants in a choice test. First larvae pupated on August 10 and were collected from the cage and kept in a Petri dish in refrigerator. In the beginning of pupation procedure, pieces of moist flower ??? were added to cages and larvae would chow tunnels into it and create pupation chambers in it. These are probably the best for shipments.

Larvae of unidentified sawfly studied as potential biological control agents for rush skeletonweed. Due to a disease, no adults were obtained for identification.


Laboratory feeding test of *Schinia cognata* on shoots of rush skeletonweed (*Chondrilla juncea*) and lettuce (Lactuca) a very closely related plants with similar size and shaped flower buds. Plants were intermixed, but larvae of *S. cognata* searched for and fed only on rush skeletonweed.

Adult of the moth *Schinia cognata* feeding on nectar of rush skeletonweed (length 15mm).


Rush skeletonweed flower head showing entry hole made by first instar larvae of *Schinia cognata*.


Open flower head of rush skeletonweed showing early instar larvae of *Schinia cognata* (length approximately 2 mm).

# Research Report on Biological Control of *Chondrilla juncea* in Bulgaria, 2007

Prof. DSc. Ivanka Lecheva, Dr. Anna Karova
Chief expert entomologist Antoaneta Petkova, Research Entomologist Cvetana Mincheva
Supervisor: Dr. Massimo Cristofaro, research Entomologist, BBCA Italy
BBCA staff: Alessandra Paolini, Francesca Lecce, Franca Di Cristina

## INTRODUCTION

The work starter after the XII International Symposium on Biological Control of Weeds, Montpellier, April 2007. Two preliminary field travels have been carried out respectively on May 9-11 and on July 6-13. During the first travel, wild plants of rush skeleton weed have been collected in two localities in Bulgaria, and transferred in pots at the University laboratory in Plovdiv and at the BBCA facilities in Rome. The second travel was focused on insect collection and on the organization of the laboratory bioassays only with *Schinia cognata*. Few *Simyrna nervosa* young instars have been collected and brought to BBCA laboratory in Rome to carry out preliminary biological observations.

A third travel to Bulgaria was carried out in late August (26-29), at the end of the experiment. During that travel some Schinia cognata mature larvae and several *Simyrna nervosa* larvae have been collected and transferred to the BBCA Italian facilities in order to carry out biological observations and preliminary host range.

## METHODS OF OBSERVATION

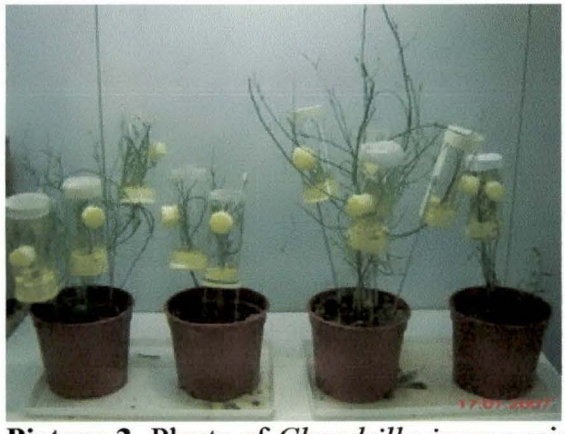
### *Schinia cognata* (Lepidoptera: Noctuidae)

Observations concerning dynamics of population density of *Schinia cognata* were carried out in an uncultivated area consisting of 6 ha located between town of Plovdiv and village of Trud, **picture 1**. Investigations were performed during the active vegetation of the host plant *Chondrilla juncea*, from April till October. The method "sweeping by entomological net" and visual method (searching leaves, branches, flower buds and flowers of host plant individually) were accomplished mostly. Observations were repeated every 10-14 days.

Examination of *S. cognata*'s larvae for verification of restricted feeding specialization of the species was conducted under laboratory conditions using folio isolators. The test was realized in the Biological Control Laboratory of Department of Entomology, Faculty of Plant Protection and Agroecology, University of Agriculture in Plovdiv, **picture 2**. The test was conducted in 2 versions, 10 replications each (first version – with 10 plants of *Chondrilla juncea*, and second one – with 10 plants of *Cichorium intybus*). In July 2007 some plants of *C. juncea* and *C. intybus* were taken from natural populations, and transferred in pots and raised under laboratory conditions, **picture 3 and 4**. Small plastic tubes placed were located on each plant in order to confine one 3rd instar and record the host range in no-choice conditions. Feeding and larval behavior were daily detected.

**Picture 1.** Uncultivated area between Plovdiv and Trud where we carried out field observations

**Picture 2.** Biological control laboratory, University of Agriculture, Plovdiv where we set the lab test



**Picture 3.** Plants of *Chondrilla juncea* raised under laboratory conditions

**Picture 4.** Plants of *Cichorium intybus* raised under laboratory conditions

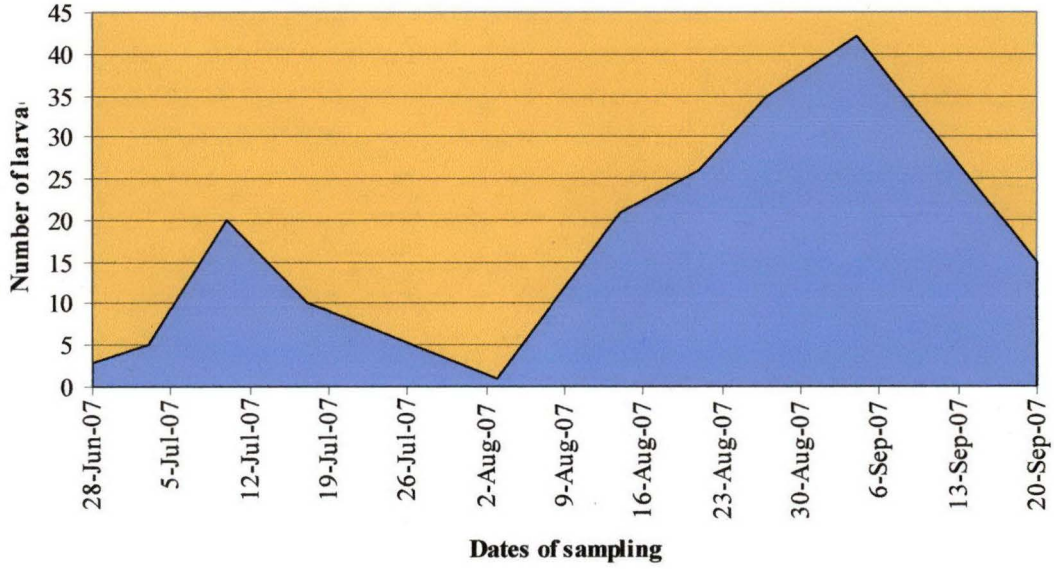
## RESULTS AND DISCUSSION

### I. POPULATION DENSITY, 2007

In 2007 the first larvae of *S. cognata* (first generation) emerged at the beginning of July – on July 3rd 5 larvae on 100 sweepings by entomological net were encountered, **figure 1**. Maximum number of larvae from first generation was reported on July 10th – 20 larvae on 100 sweepings by entomological net. All larvae of first generation pupated normally despite of the extremely high temperatures that took place in July 2007.

The first larvae of the second generation appeared on August 3rd. September turned out to be unusually favorable for the development of *S. cognata* larvae. From the beginning of the month till September, 20th, really high density of the caterpillars was recorded. Maximum number of larvae from second generation was reported on September, 4th, when 42 caterpillars were encountered. Larval density decreased after September, 20th, **fig. 1**.

**Fig. 1. Dinamics of population density of larvae of *Schinia cognata* in *Chondrilla juncea* population in the region of Plovdiv, 2007**




### II. EXPERIMENT FOR VERIFICATION OF RESTRICTED FEEDING SPECIALIZATION

In the version with *Ch. juncea* in all 10 replications each larva was feeding as usual and developed normally into a pupa and then reared to an adult, **picture 5 and 6.**

**Picture 5.** Larva of *Sch. cognata* feeding on *Ch. juncea* flower in isolator

**Picture 6.** Larva of *Sch. cognata* turned into a pupa in isolator placed on *Ch. juncea* plant

In the version with *C. intybus* in all 10 replications not even one larva did feed on the plant. All larvae were wandering about permanently for the first 2 days but after that they began to die. 7 days after the experiment was set all larvae were dead because they refused to feed however they were provided enough food, **picture 7.**

**Picture 7.** Dead larvae of *S. cognata* in isolator, placed on *C. intybus* plant

***Simyna nervosa* (Lepidoptera: Noctuidae)**

As a result of our observations it was found that the noctuid moth *Simyrna nervosa* develops two generations per year.

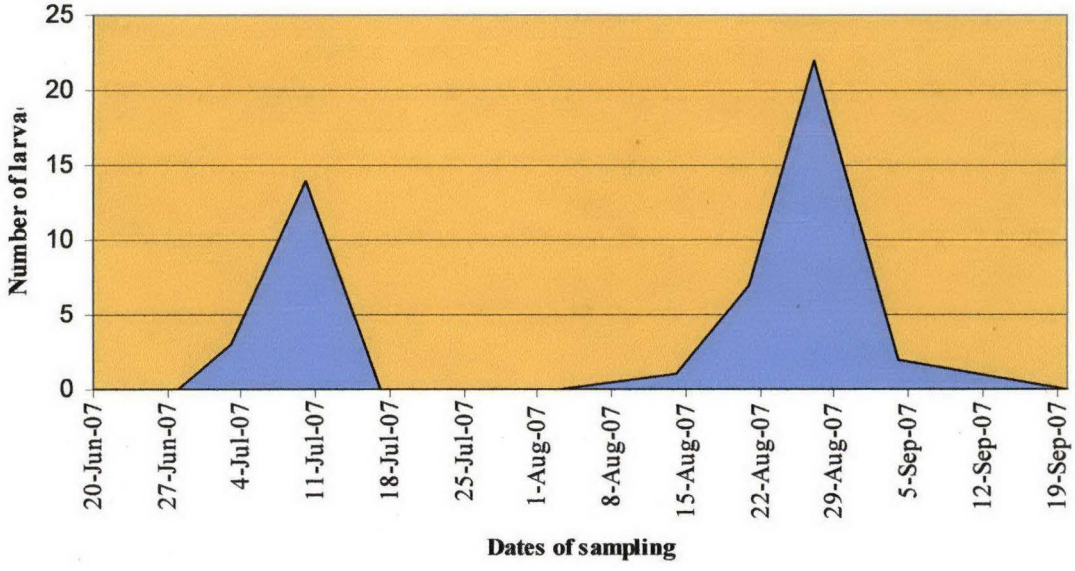
FOR THE CONDITIONS OF PLOVDIV in 2007 the first generation began its development during the period of the end of June and beginning of July. During 2007, first larvae were detected, on July, 3rd. They were feeding openly on the flower heads of *Chondrilla juncea*, **picture 8.** At the next observation, on July, 10th, caterpillars' density was considerably higher – 14 caterpillars, 10 of which were 5th instar and 4 of them - 3rd. Larvae pupated at the end of July.

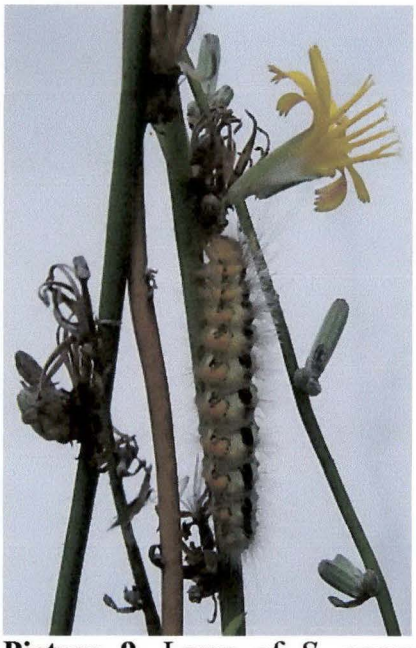
The second generation was recorded starting from mid August. Final field observations carried out at the end of August showed a high density for all the instars, **figure 2.**

*Simyrna nervosa* ended its life cycle in field condition in Bulgaria at the beginning of September.

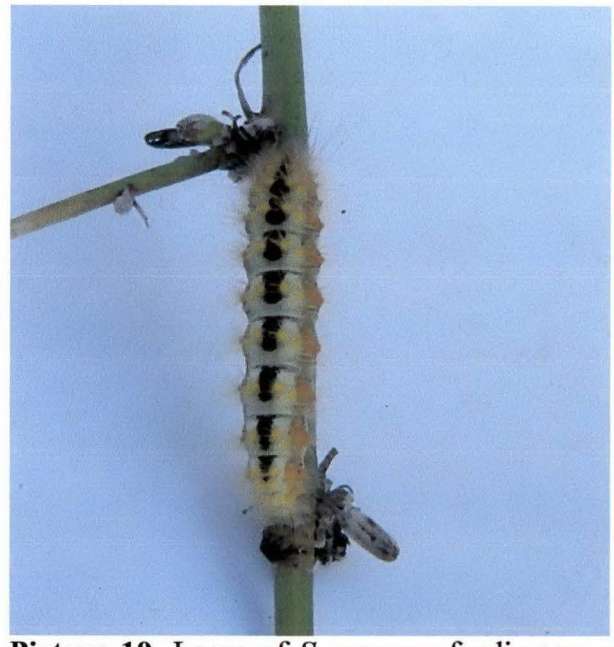
**Picture 8.** Larva of *Simyrna nervosa* feeding openly on the flower heads of *Chondrilla juncea*

**Fig. 2. Dinamics of population density of larvae of *Symira nervosa* in *Chondrilla juncea* population in the region of Plovdiv, 2007**





**Picture 9.** Larva of *S. nervosa* feeding openly on the flowers of *Ch. juncea*



**Picture 10.** Larva of *S. nervosa* feeding openly on the stem of *Ch. juncea*

## LABORATORY TESTS AT BBCA FACILITIES, ROME, ITALY.

A first group of *Simyna nervosa* larvae, collected in Bulgaria during the field trip in mid July, have been reared and used for preliminary host range observations in choice condition. Starting from July 17, four test plants (RSW, chicory, lettuce and *Aster chilensis*), prepared in bouquets, were offered to 2 *S. nervosa* larvae, confined in a transparent plastic sleeve cage(30x30x30 cm). Bouquets, located at the 4 corners of the cage bottom, were changed every day. A total of 6 replications were carried out. A second host range choice test has been carried out starting on August 31. In this case, 3 replications have been carried out confining 2 larvae in a transparent plastic cage with 2 bouquets: a bouquet of RSW and a bouquet of another of 3 other test plants (chicory, artichoke, *Cirsium hidrophyllum*). The test was closed on Sept 05. The third and last choice host range test was carried out in October, using the progeny of the previous tests. In this case the test was similar to the second test (choice with 2 plants, RSW and another test plant: chicory, *Aster chilensis*, artichoke, and field bindweed). As for the others, 2larvae (in this case neonate) were confined in the cage and feeding and longevity was daily recorded.

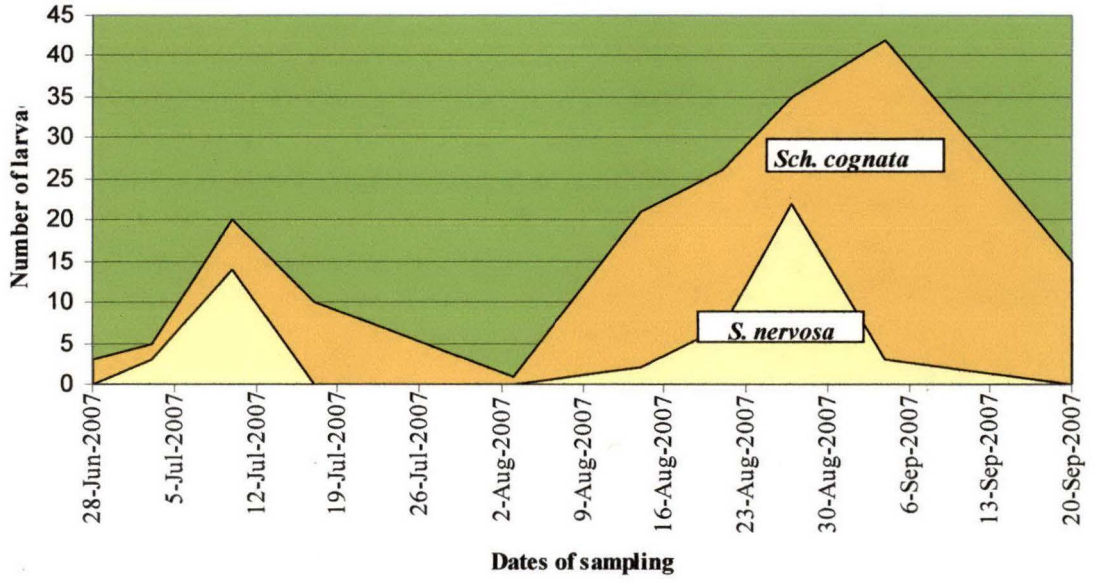
## RESULTS

In all the laboratory tests *Simyrna* did not showed a specific feeding behavior. Larvae were able to feed on all the test plants we offered, excluding the "real outgroup" field bindweed (which is not an Asteracea, but a Convolvulacea).Very often we recorded the feeding only on one plant species, but the next day the same larvae were shifting to another host. Pupation rate was relatively high, and several pupae are in diapause at the BBCA facilities.

## CONCLUSION

However the summer season of 2007 had extremely hot temperatures and no rains fell down larvae of both noctuid moths *Schinia cognata* and *Symira nervosa* developed normally and were observed at really high density, **figure 3**.

**Fig.3. Dinamics of population density of larvae of noctuid moths *Schinia cognata* and *Symira nervosa* in *Chondrilla juncea* population in the region of Plovdiv, 2007**




In conclusion we would say that the experiment for verification of the feeding specialization of larvae of *S. cognata* once again proves categorically that the species demonstrates restricted feeding specialization and feeds only on *Chondrilla juncea*. If the host plant is not available larvae die in some days.

Despite to the fact we observed a "poor" host specificity in laboratory conditions, we suggested to repeat again the tests with *Simyrna*. In addition, it is really important before to "declare" correct our data, to receive the taxonomic response for the adults reared in our laboratory from the field collected larvae and used during the tests at the BBCA facilities. An interesting aspect that must be taken under consideration is the fact that in field conditions *S. nervosa* was always found only on *Chondrilla juncea*. This kind of "conflict" between field observations and laboratory data, can be due to the eventual specificity of the oviposition behavior, or could be attributed to a "wrong" unknown parameter in the laboratory setting. For this reason, we recommend to repeat the tests with *Simyrna nervosa*, next year both in laboratory and in field conditions.

# Field and laboratory studies on biology and host specificity of *Sphenoptera foveola* (Gebler) (Coleoptera, Buprestidae), potential agent for biological control of the rush skeleton weed, *Chondrilla juncea* L, conducted by Biocontrol Group (Zoological Institute, St.Petersburg, Russia) in 2007

## SUMMARY

During 2007, the following work was conducted in Kazakhstan, in Russia, and in Armenia:

1) Reading of the results of the earlier (in 2005) started field tests on host specificity, collecting adults for new field and laboratory studies, and conducting selective field sampling in natural habitats of *S. foveola* in Almaty region of Kazakhstan.
2) Establishing and reading of the results of field tests on host specificity in Rostov prov. of Russia.
3) Establishing and reading of the results of field tests on host specificity in Armenia.
4) Laboratory experiments on host specificity conducted in laboratory facilities of Biocontrol Group (St. Petersburg, Russia).

During these studies, new important data were obtained. In combination, these data suggest that *S. foveola* host specificity is very strict. It is definitely limited by species of the genus *Chondrilla* and, moreover, it seems that *S. foveola* could successfully develop only on a certain group of *Chondrilla* species. Some data obtained in field and in laboratory conditions suggest that the target weed, *Chondrilla juncea*, in certain cases can be also suitable for adult and larval feeding. Particularly, *S. foveola* adult feeding on *Ch. juncea* was first recorded in test plants in natural conditions in Russia and *S. foveola* larva feeding on *Ch. juncea* was first recorded in test plant in natural conditions in Armenia. In addition, larval feeding on *Ch. graminea,* which is closely related to *Ch. juncea* was recorded in laboratory. On the other hand, it seems that both in field and in laboratory conditions, only few of the tested individuals were able to develop when feed on *Ch. juncea* roots which could be possibly considered as a result of intraspecific variability. Thus, the further studies are necessary to finally investigate the host specificity of *S. foveola* ovipiosition, larval feeding, and development and to estimate the potential of this buprestid for biological control of the rush skeleton weed.

## Field collection of adults for laboratory tests

About 140 *S. foveola* adults were collected from or under *Ch. ambigua* and *Ch. canescens* plants during 19-24 June 2007 in their natural habitats (Fig. 1) in Qumbasy sandy desert, 69 km NNW of Qapshaghay, Almaty Province, Kazakhstan (ca 44°25'34" N and 76°47'42" E) and were later used for field and laboratory tests.

Fig. 1. Places where *Sphenoptera foveola* adults were collected (Kazakhstan, June, 2007)

## Field observations on host specificity

With this aim, roots of plants from several species of Asteraceae family, naturally growing in close vicinity to *Chondrilla* plants infested by *S. foveola* were drawn out and inspected for *S. foveola* larvae or any traces of their feeding, i.e. root galls (more exactly, latex cases). This sampling was conducted in the same habitats, where adults for filed and laboratory tests were collected. Several sites with various latex containing and not containing xerophilous plant species were inspected, plants for sampling were selected randomly by squares or along transects. The results are summarized in table 1. From our data, it is seen that (at least in the studied population and habitats) *S. foveola* larvae could be found only on *Chondrilla* species. As for the difference between *Ch. ambigua* (sect. Brachyrhynchus) and *Ch. canescens* (sect. Euchondrilla), the two species seems to be more or less equally populated, the difference being insignificant, at least at the given sample sizes.

Table 1. *Sphenoptera foveola* host specificity in natural conditions (selective sampling)

| Plant species | Number inspected | Number of plants with traces of *S. foveola* : | | | Total root galls, percentage |
|---|---|---|---|---|---|
| | | empty root galls | root galls with larvae | total root galls | |
| *Lactuca* sp.?* | 10 | 0 | 0 | 0 | 0 % |
| *Scorzonera* sp.? * | 16 | 0 | 0 | 0 | 0 % |
| *Sonchus* sp.? * | 31 | 0 | 0 | 0 | 0 % |
| *Echinops* sp. | 20 | 0 | 0 | 0 | 0 % |
| *Jurinea* sp. | 12 | 0 | 0 | 0 | 0 % |
| *Cousinia* sp. | 23 | 0 | 0 | 0 | 0 % |
| *Centaurea diffusa* Lam. | 4 | 0 | 0 | 0 | 0 % |
| *Chondrilla canescens* Kar.&Kir.* | 52 | 22 | 15 | 37 | 71% |
| *Chondrilla ambigua* Fisch. * | 133 | 89 | 18 | 107 | 80% |

*) Plant species containing latex

## Field tests on host specificity

1) **Field tests in Kazakhstan**

In June 2007, the results of no-choice field tests established in Kazakhstan in 2005 were finally recorded. This work was aimed to evaluate host specificity (oviposition and larval development) of *S. foveola* in no-choice tests under natural conditions. In each test, one naturally growing plant was used. The test plant was covered by cage made of cotton and gauze (Fig. 2). In most of cages, one *S. foveola* male and one female were placed, but only females were used in a few cases. Inside the cage, the soil was covered with sand to offer a substrate suitable for oviposition. The base of the cage was fixed in soil. Position of each test plant was recorded by GPS with the most possible accuracy, which made possible to locate almost each test plant 2 years later. Most of test plants turned to be died and completely destroyed, but in some other cases the reading of the results was possible. In a few cases, the dead beetles were found inside the cages.

From table 2 it is seen that most of test plants died more or less independently of the plant species which was possibly caused by too late removing of cages (when this experiment was started, it was planned to remove the cages in 2005). It seems that the cage itself physically depressed the plant when it was covered during more than 2 years or may be the cage promoted development of some insect pests as e.g. aphids. Anyway, basing on the data obtained (table 2), it is clear that remnants of root galls were found only on plants

of Chondrilla species, and in none of other tested plants. However, all of the galls found on test plants were empty: obviously, larvae have finished feeding and have pupated during two years passed since the beginning of the test. Note that in similar galls on control plants of the same *Chondrilla* species, *S. foveola* larvae were found (fig. 3).

**Fig. 2.** Mounting cages for *S. foveola* no-choice host range tests in natural conditions (Kazakhstan, 2005).

**Fig. 3.** Reading of the results of *S. foveola* no-choice host range tests in natural conditions in Kazakhstan: opening the cage, digging the root, opening the galls, dissected gall with mature larva from control plant (Kazakhstan, 2007)

Table 2. *Sphenoptera foveola* oviposition and larval development no-choice tests in natural conditions (Kazakhstan).

| Cage ID | Coordinates | Host Plant | Results |
|---|---|---|---|
| S-01 | 44.21.16,3 N 76.57.52,3 E | *Scorzonera* sp. ? | Destroyed plant, no galls |
| S-02 | 44.21.16,3 N 76.57.52,4 E | *Scorzonera* sp.? | Destroyed plant, no galls |
| S-03 | 44.21.17,1 N 76.57.55,8 E | *Scorzonera* sp.? | Not found |
| S-04 | 44.21.15,8 N 76.57.56,6 E | *Scorzonera* sp.? | Destroyed cage |
| S-05 | 44.21.15,2 N 76.57.56,2 E | *Scorzonera* sp.? | Destroyed cage |
| S-06 | 44.21.15,2 N 76.57.56,6 E | *Cousinia* sp. | Not found |
| S-07 | 44.21.14,8 N 76.57.56,8 E | *Cousinia* sp. | Not found |
| S-08 | 44.21.13,8 N 76.57.58,5 E | *Cousinia* sp. | Not found |
| S-09 | 44.21.13,1 N 76.57.58,8 E | *Cousinia* sp. | Destroyed plant, no galls |
| S-10 | 44.21.11,9 N 76.57.59,8 E | *Cousinia* sp. | Reamnants of beetles, destroyed plant, no galls |
| S-11 | 44.21.11,4 N 76.57.58,0 E | *Taraxacum* sp. | Not found |
| S-12 | 44.21.11,2 N 76.57.56,8 E | *Taraxacum* sp. | Destroyed plant |
| S-13 | 44.21.10,5 N 76.57.56,6 E | *Taraxacum* sp. | Destroyed plant |
| S-14 | 44.21.09,7 N 76.57.56,2 E | *Taraxacum* sp. | Destroyed plant |
| S-15 | 44.21.09,0 N 76.57.55,2 E | *Taraxacum* sp. | Destroyed cage |
| S-16 | 44.21.20,1 N 76.58.01,1 E | *Chondrilla canescens* | Destroyed plant |
| S-17 | 44.21.19,9 N 76.58.00,9 E | *Chondrilla canescens* | Not found |
| S-18 | 44.21.20,5 N 76.58.02,0 E | *Chondrilla canescens* | Not found |
| S-19 | 44.21.22,0 N 76.58.01,7 E | *Chondrilla canescens* | Destroyed plant |
| S-20 | 44.21.21,4 N 76.58.06,4 E | *Chondrilla canescens* | Not found |
| S-21 | 44.25.48,5 N 76.47.35,9 E | *Chondrilla canescens* | Remnants of root galls |
| S-22 | 44.25.48,1 N 76.47.37,0 E | *Chondrilla canescens* | Destroyed plant |
| S-23 | 44.25.47,7 N 76.47.36,4 E | *Chondrilla canescens* | Destroyed plant |
| S-24 | 44.25.48,5 N 76.47.36,8 E | *Chondrilla canescens* | Young plant, remnants of old root galls |
| S-25 | 44.25.48,0 N 76.47.36,8 E | *Chondrilla canescens* | Dead beetles, remnants of root galls |
| S-26 | 44.25.45,9 N 76.47.21,0 E | *Chondrilla ambigua* | Destroyed plant |
| S-27 | 44.25.46,5 N 76.47.16,8 E | *Chondrilla ambigua* | Destroyed plant |
| S-28 | 44.25.43,3 N 76.47.13,0 E | *Chondrilla ambigua* | Remnants of root galls |
| S-29 | 44.25.43,1 N 76.47.11,9 E | *Chondrilla ambigua* | Destroyed plant |
| S-30 | 44.25.42,6 N 76.47.04,3 E | *Chondrilla ambigua* | Not found |

Thus, we conclude that in their natural habitats (sandy deserts of Kazakhstan) *Sphenoptera foveola* larvae develop only on plants of the genus *Chondrilla* (particularly, *C. ambigua* and *C. canesces*), the difference between the last two species being insignificant, al least based on the studied samples. Note that this conclusion was made both from "observations" (selective sampling and inspection of different plant species, table 1) and from "experiments" (no-choice tests of oviposition and larval feeding specificity, table 2).

2) **Field tests in Russia**

Field tests in Southern Russia (Kalitvenskaya village, environs of Kamensk, Rostov province), were established at 5.07.2007 in the population with ca 100 *Chondrilla juncea* plants naturally growing in sandy clearing of mixed forest The cages were mounted by the same method which was used in Kazakhstan (see fig. 2) and S. foveola adults collected in Kazakhstan (Qumbasy location) were used. In these tests only native *Chondrilla juncea* were used as a host plant. In ca 20 days (31.07.2007), the cages were opened and removed from test plants. At the same time, test plants were checked for traces of adult feeding (Fig. 4). Three months later, 15.10.2007, roots of all test plants were drawn out and inspected for any traces of larval feeding. The results of this experiment are presented in table 3. It is seen that the feeding traces were recorded in more than a half of the cages suggesting that *Ch. juncea* is suitable for *S. foveola* adult feeding. However, in none of the tested plants any traces of larval feeding were observed.

Fig. 4. *S. foveola* no-choice host range tests in natural conditions in Russia: cage on the plant, removed cage near the plant, traces of adult feeding (Kamensk, 31.07.2007).

Table 3. *Sphenoptera foveola* oviposition and larval development no-choice tests in natural conditions (Russia).

| Cage ID | Coordinates | Host Plant | Adult feeding (traces) | Larvae feeding |
|---|---|---|---|---|
| R01 | 48.15.02 N 40.28.44 E | Ch. juncea | Yes | Healthy plant, no traces of larval feeding |
| R02 | 48.15.02 N 40.28.44 E | Ch. juncea | Yes | Healthy plant, no traces of larval feeding |
| R03 | 48.15.02 N 40.28.44 E | Ch. juncea | | Destroyed plant |
| R04 | 48.15.02 N 40.28.44 E | Ch. juncea | No | Healthy plant, no traces of larval feeding |
| R05 | 48.15.02 N 40.28.42 E | Ch. juncea | Yes | Healthy plant, no traces of larval feeding |
| R06 | 48.15.02 N 40.28.42 E | Ch. juncea | | Destroyed plant |
| R07 | 48.15.02 N 40.28.42 E | Ch. juncea | No | Healthy plant, no traces of larval feeding |
| R08 | 48.15.02 N 40.28.42 E | Ch. juncea | Yes | Healthy plant, no traces of larval feeding |
| R09 | 48.15.02 N 40.28.42 E | Ch. juncea | | Destroyed plant |
| R10 | 48.15.02 N 40.28.40 E | Ch. juncea | No | Healthy plant, no traces of larval feeding |
| R11 | 48.15.02 N 40.28.40 E | Ch. juncea | Yes | Healthy plant, no traces of larval feeding |
| R12 | 48.15.02 N 40.28.40 E | Ch. juncea | Yes | Healthy plant, no traces of larval feeding |
| R13 | 48.15.02 N 40.28.40 E | Ch. juncea | Yes | Healthy plant, no traces of larval feeding |
| R14 | 48.15.02 N 40.28.40 E | Ch. juncea | Yes | Healthy plant, no traces of larval feeding |

3) **Field tests in Armenia**

Similar no-choice field tests were conducted in 2007 in two locations in Armenia: Uranots near Surenavan (ca 39.44 N, 44.49 E) and Goravan (Vedi environs, ca 39.55 N, 44.46 E ). Same as in Russia, only naturally grown *Chondrilla juncea* plants were used. In two sites, 20 cages were established by the method described above also using *S. foveola* adults from Kazakhstan (Qumbasy location. The difference between sites was in the type of soil. In the first site (Surenavan), it was rather stony, while in the second site (Goravan), it was almost pure sand (Fig. 5). In three months after the beginning of the test, roots of all tested plants were inspected. In addition, other 10 "control" naturally growing plants were studied. In none of these control plants any buprestid larvae was recorded, while in one of tested plants growing in sandy site *Sphenoptera* larva was found (Fig. 5).

Fig. 5. No-choice specificity tests in Armenia: stony (Surenavan) and sandy (Gorava) deserts, roots prepared for dissection and *Sphenoptera* larva found in Goravan location in one of tested *Ch. juncea* plants.

**Laboratory tests**

For laboratory tests, eggs laid by females collected in Kazakhstan during their transportation to St.Petersburg were used. In addition, the rest of adults which were not used for the field tests were kept in laboratory cage fed with *Ch. juncea* plants (Fig. 6, 7). Eggs found were collected and placed in Petri dishes filled with slightly moist sand or covered with slightly moist filter paper. *S. foveola* larvae successfully eclosed under both conditions, and neonate larvae were used for no-choice host specificity tests with potted plants. All plants used in these tests were grown from seeds in laboratory conditions. For each test, 3-5 larvae were gently transferred by soft brush into the sandy soil close to the stem base of the tested potted plant. In approximately a month, root of each tested plant was dissected and inspected for *S. foveola* larvae or any traces of their feeding (table 4). Larvae or traces of larval feeding were found only on species of the genus *Chondrilla* (Fig. 8).

Fig. 6. The cage with *Sphenoptera foveola* females in laboratory.

Fig. 7. *Sphenoptera foveola* female feeding *Chondrilla juncea* in laboratory conditions

Fig. 8. *Sphenoptera foveola* larvae feeding on *Chondrilla ambigua* roots and root galls (latex cases) in laboratory conditions

Table 4. *Sphenoptera foveola* larvae host specificity no-choice tests in laboratory conditions.

| Host plant species | Number of tested | | Number of positive results (at least one larva or traces of larval feeding found) | | | Percentage of larvae survived |
|---|---|---|---|---|---|---|
| | plants | larvae | plants | larvae | traces of larval feeding | |
| *Taraxacum* sp. | 13 | 49 | 0 | 0 | 0 | 0 |
| *Sonchus* sp.? | 8 | 25 | 0 | 0 | 0 | 0 |
| *Chondrilla graminea* Bieb. * | 7 | 21 | 2 | 0 | 2 | 0 |
| *Chondrilla juncea*[1] | 7 | 20 | 0 | 0 | 0 | 0 |
| *Chondrilla juncea*[2] | 2 | 6 | 0 | 0 | 0 | 0 |
| *Chondrilla juncea*[3] | 1 | 3 | 0 | 0 | 0 | 0 |
| *Chondrilla juncea*[4] | 1 | 3 | 0 | 0 | 0 | 0 |
| *Chondrilla ambigua* Fisch. ** | 5 | 25 | 4 | 7 | 0 | 28% |

* grown from seeds originated from Volgograd province, Russia

1) – grown from seeds originated from Roggins (Idaho, USA)

2) – grown from seeds originated from Bantis (Idaho, USA)

3) – grown from seeds originated from Lime bay, Anderson Reservoir (Idaho, USA)

4) – grown from seeds originated from Farrigut State Park (USA)

** grown from seeds originated from Kazakhstan

## Conclusions

Summarizing the results, we conclude that *S. foveola* host specificity is very strict. It is definitely limited by species of the genus *Chondrilla* and, moreover, it is obvious from field observations in Kazakhstan that *S. foveola* could successfully develop only on a certain group of *Chondrilla* species from Brachyrhynchus (*Ch. ambigua*) and Euchondrilla (*Ch. canescens*) sections. Some data obtained in field and in laboratory conditions suggest that the target weed, *Chondrilla juncea* (sect. Euchondrilla) can be also suitable for adult and larval feeding. Particularly, *S. foveola* adult feeding on *Ch. juncea* was first recorded in natural conditions in Russia (fig. 4), and presumably *S. foveola* larvae feeding on *Ch. juncea* was first recorded in natural conditions in Armenia (fig. 5). In addition, larval feeding on *Ch. graminea,* which is closely related to *Ch. juncea* was recorded in laboratory (table 4). On the other hand, it seems that both in field and in laboratory conditions, only few of the tested individuals were able to develop when feed on *Ch. juncea* roots. Note that this could not be explained by low survival, as e.g. in laboratory tests almost all of plants belonged to *Chondrilla* species native to Kazakhstan gave positive results (table 4). Possibly, this could be a result of intraspecific variability. Thus, the further studies are necessary to finally investigate the host specificity of *S. foveola* ovipiosition, larval feeding, and development and to estimate the potential of this buprestid for biological control of the rush skeleton weed.

# Field and laboratory studies on biology and host specificity of *Sphenoptera foveola* (Gebler) (Coleoptera, Buprestidae), potential agent for biological control of the rush skeleton weed, *Chondrilla juncea* L, conducted by Biocontrol Group (Zoological Institute, St.Petersburg, Russia) in 2007

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November 11, 2007

## SUMMARY

During 2007, the following work was conducted in Kazakhstan, in Russia, and in Armenia:

1) Reading of the results of the earlier (in 2005) started field tests on host specificity, collecting adults for new field and laboratory studies, and conducting selective field sampling in natural habitats of *S. foveola* in Almaty region of Kazakhstan.
2) Establishing and reading of the results of field tests on host specificity in Rostov prov. of Russia.
3) Establishing and reading of the results of field tests on host specificity in Armenia.
4) Laboratory experiments on host specificity conducted in laboratory facilities of Biocontrol Group (St. Petersburg, Russia).

During these studies, new important data were obtained. In combination, these data suggest that *S. foveola* host specificity is very strict. It is definitely limited by species of the genus *Chondrilla* and, moreover, it seems that *S. foveola* could successfully develop only on a certain group of *Chondrilla* species. Some data obtained in field and in laboratory conditions suggest that the target weed, *Chondrilla juncea*, in certain cases can be also suitable for adult and larval feeding. Particularly, *S. foveola* adult feeding on *C. juncea* was first recorded in test plants in natural conditions in Russia and *S. foveola* larva feeding on *C. juncea* was first recorded in test plant in natural conditions in Armenia. In addition, larval feeding on *C. graminea,* which is closely related to *C. juncea* was recorded in laboratory. On the other hand, it seems that both in field and in laboratory conditions, only few of the tested individuals were able to develop when feed on *C. juncea* roots which could be possibly considered as a result of intraspecific variability. Thus, the further studies are necessary to finally investigate the host specificity of *S. foveola* ovipiosition, larval feeding, and development and to estimate the potential of this buprestid for biological control of the rush skeleton weed.